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Genomic control of receptor function

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Abstract. Ionotropic ligand-gated channels constitute a large superfamily of channels that provide a molecular basis for synaptic transmission in central and peripheral nervous systems. These channels are subjects of genomic regulation at different levels. The final functional properties of each particular channel are determined by type of gene family, posttranscriptional alterations of messenger RNA (alternative splicing, editing) and subunit composition. In addition, interaction of receptor subunits with postsynaptic density proteins plays a regulatory role and determines targets of channel insertion. In this review, taking glutamate receptor channels as well-studied example, we illustrate how each of these steps may determine receptor function in synapses.

Key words. Ionotropic glutamate receptor channels; alternative splicing; mRNA editing; synaptic transmission.

Introduction

Ionotropic ligand-gated channels activated by excitatory neurotransmitters: glutamate, acetylcholine, ATP or serotonin—and inhibitory neurotransmitters γ-amino butyric acid (GABA) or glycine play a key role in synaptic transmission in the mammalian central nervous system (CNS). Molecular cloning exposed a large variety of the ligand-gated channel subunits differentially expressed in mammalian brain. Functional analysis of cloned and native receptors has revealed different levels of genomic control of the receptor functions. First of all, a variety of receptor subunits are encoded by different genes. This subunit diversity is further multiplied by posttranscriptional alterations such as alternative splicing and messenger RNA (mRNA) editing. In addition, individual subunits may combine in heteromeric assemblies to form functional receptors with distinct functional properties. Finally, receptor subunits express in the brain in a cell- and age-specific manner. Thus, the functional property of the receptors mediating synaptic transmission in a particular neuronal circuit is a product of multiple genetically controlled transformations. Several reviews have appeared that describe in great detail structural peculiarities of different ionotropic receptors and the mechanisms underlying their functional diversity [1–8]. In this article we highlight some of the important studies on ionotropic glutamate receptors (GluRs), outlining the levels of genomic control of the receptors structure and function (which appears to be common for different ligand-gated channels) and describing the molecular background of the receptor diversity and in particular how this diversity is exploited in functioning synapses.

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Diversity of ionotropic GluR channels

Ionotropic GluR channels mediate excitatory synaptic transmission in most regions of the mammalian CNS. GluR subunits are encoded by at least 15 different genes grouped in three families according to sequence homology and preferential binding to more specific agonists. These are α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptors (AMPAR: GluR1 to 4 or GluR-A to -D), kainate receptors (KAR: GluR5 to 7, KA1 and KA2) and *N*-methyl-D-aspartate receptors (NMDAR: NR1, NR2A to 2D and NR3A) [1, 9]. The structural subunit diversity between families underlies diversity in function of the channels formed by these subunits. Besides different affinity to specific agonists and the existence of specific antagonists, the general differences between subunit families are in the biophysical properties of the channels. Thus, channels composed

of AMPAR and KAR subunits mediate glutamate-evoked whole-cell currents with fast onset, deactivation and desensitization kinetics, whereas the kinetics of the currents mediated by channels composed of NMDAR subunits are much slower [10]. In most of the CNS glutamatergic synapses AMPARs and NMDARs are colocalized at the same postsynaptic densities, resulting in a dual component of the synaptic response. The fast component is mediated by AMPARs, and the slow component is mediated by NMDARs [11, 12]. Another fundamental difference between NMDAR and non-NMDAR subunit channels is that the NMDAR channels are more permeable to Ca^{2+} and undergo voltage-dependent block by extracellular Mg^{2+} at physiological concentrations [13–15].

A further dimension of the functional diversity of GluRs within families results from posttranscriptional alterations of the subunit structure by alternative splicing and mRNA editing.

Alternative splicing in GluR subunits

In each subunit of the AMPAR family a segment of 38 amino acid residues preceding the predicted fourth transmembrane region (M4) exists in one of two sequence versions. The exchange of small homologous domains in four glutamate receptors suggested that two receptor versions for each of the four family members, named 'flip' and 'flop', arose from alternative splicing [16]. Several splice variants of C-terminals were found for GluR-B and GluR-D subunits [17, 18] and also for other GluR subunits: GluR5, GluR6 and GluR7 of the KAR family [19–21], and NR1 of the NMDA receptor family [22, 23]. Sequence differences due to splicing

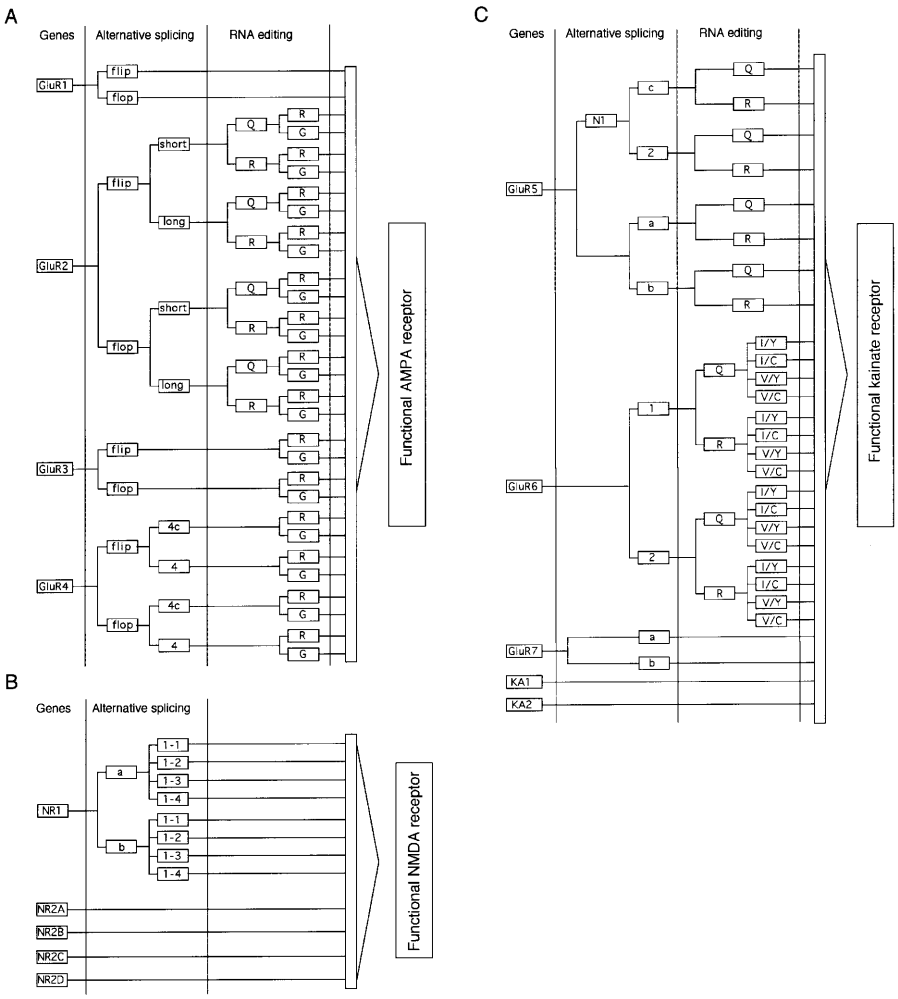

Figure 1. Subunit diversity of GluR channels. Each box represents a subunit variant of AMPARs (*A*), NMDARs (*B*) and rat KARs (*C*) altered by either alternative splicing or mRNA editing. Nomenclature as in [3].

occur also in the N-terminal extracellular domains of GluR5 and NR1 subunits [20, 23, 24] (fig. 1).

mRNA editing of GluR subunits

The primary structure of some of the AMPAR and KAR subunits can be modified by mRNA editing. In GluR-B, GluR5 and GluR6 subunits the site (Q/R site) that forms a selectivity filter in the pore lining M2 region glutamine (Q) may be replaced by arginine (R) [25]. This arises from a single nucleotide exchange within the first glutamine codon (CAG to CGG). In addition, GluR-B-D subunits might be edited in the so-called R/G site in the loop between the M3 and M4 regions. In this site the first adenosine of the arginine codon (AGA) is changed to guanosine to form a glycine (G) codon (GGA) [26]. The KAR subunit GluR6 may be additionally edited in two sites in the M1 region, producing subunits with isoleucine (I, codon ATT) or valine (V, codon GTT) in one site and tyrosine (Y, codon TAC) or cysteine (C, codon TGC) in the other [27]. Interestingly, the level of editing for all editing sites depends, although differently, on the developmental stage. Thus, $>99\%$ of GluR-B subunits in rats are edited in the Q/R site postnatally [28]. In contrast, different ratios of edited and unedited variants at postnatal ages are found for the R/G-site of GluR-B-D subunits and for the KAR subunits GluR5 (Q/R site) and GluR6 (Q/R, I/V and Y/C sites) [26, 29].

mRNA editing

Perhaps the most fascinating functional consequences result from mRNA editing. Substitution of one amino acid (Q to R) in the pore-forming M2 domain in homomeric AMPAR and KAR channels dramatically reduces Ca^{2+} permeability, single-channel conductance, sensitivity to block by endogenous intracellular polyamines and affects cation versus anion permeability [32–36]. The mRNA for the unedited Q form of the GluR-B subunit is expressed in an appreciable amount (up to 20%) in brain only during early embryonic development [28]. Recent study [37] has shown that the absence of Q/R-site editing of the GluR-B subunit does not alter functioning of AMPAR channels in mouse brain. Mice that carry in their GluR-B alleles a Q-to-R codon substitution for the Q/R site showed normal development and no obvious deficiencies. This suggests that the functional significance of this naturally occurring mutation for rapid AMPAR-mediated synaptic transmission arises rather from the fact that coexpression of the edited GluR-B subunit with one of the other AMPAR subunits may lead to formation of AMPAR channels with a wide spectrum of the properties. Editing in the M1 region of the KAR GluR6 subunit also alters Ca^{2+} permeability of the homomeric channels, but to a lesser extent [27]. Editing in the R/G site of AMPAR channels affects the desensitization properties of the receptors, resulting in a lesser extent of desensitization and a faster recovery from desensitization as measured using the paired-pulse protocol [26].

Consequences of the posttranscriptional alterations to functional properties of GluR subunits

Alternative splicing

It has been shown in recombinant systems using rapid application of glutamate that currents through AMPAR flip and flop splice variants differ in desensitization kinetics. Flop forms of subunits have a two- to fourfold faster time course and more complete desensitization than the flip forms. In the GluR-D_{flop} subunit, for instance, current desensitization kinetics proceeds as rapidly as the deactivation time course [30]. For other subunits the latter is typically faster than the desensitization time course. It was recently shown that homomeric KAR channels formed from GluR7a subunit splice variant mediate approximately sevenfold larger glutamate-evoked currents with a faster rise time than those of GluR7b subunit splice variants [21]. In the NMDAR NR1 subunit C1 cassette present in NR1-1 and NR1-3 splice variants and which contains one of the binding sites for calmodulin, at least in part, contributes to Ca^{2+}-dependent inhibition of heteromeric NMDAR channels [31].

Subunit composition and its implication for receptor function

Studies of cloned channels revealed functional consequences of the posttranscriptional genomic regulations for the most of the subunits at a subunit level. However, even when the functional signature of the individual subunit is known, it is still problematic to directly correlate the functional properties of recombinant channels to those in neuronal synapses. Most of the GluRs in the synapses are likely composed of more than one subunit type. This arises from the fact that GluR subunits within subfamilies may coexpress to form heteromeric channels. The coexpression of subunits in neuronal membranes occurs in unknown combination. Simple calculation of a number of theoretically possible combinations of channel assembly within, for example, the AMPAR subfamily, gives an enormously large value ($>100{,}000$), assuming a pentameric structure of the channel). Perhaps not all combinations of subunits would result in dramatic functional differences of the assembled channels. Nevertheless, the existence of a particular subunit in an ensemble channel may deter-

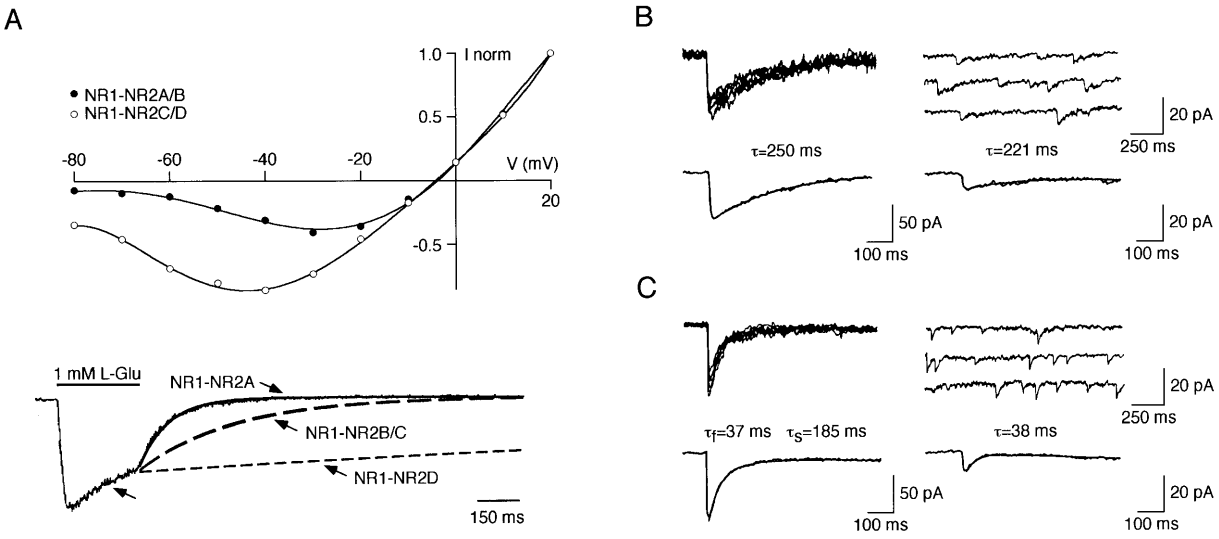

Figure 2. Functional distinctions of NMDAR subunits underlie diversity of synaptic response. (*A*) Identity of the NR2 subunits determines sensitivity to extracellular Mg^{2+} (1 mM) and voltage dependence of the Mg^{2+} block in heteromeric NMDAR channels. Current-voltage relations are normalized to the current amplitudes at 20 mV (top). Offset kinetics of glutamate-activated whole-cell currents through recombinant NMDAR channels is subunit dependent. Dotted lines represent exponential fits of the current deactivation for indicated subunit combinations. Data obtained in whole-cell mode from HEK 293 cells transfected with different subunit combinations (bottom). (*B*, *C*) Developmental switch in kinetics of synaptic NMDAR-mediated currents in layer IV neurons. (*B*) Overlaid evoked NMDAR-mediated currents measured from 12-day-old rats (left upper traces) and averaged trace fitted by single exponential (bottom left). Individual spontaneous NMDA-EPSCs (right upper traces) and average of 20 consecutive events (left bottom trace). (*C*) The same as in *B* but measured from layer IV neurons of 35-day-old rats. The averaged trace (left bottom) was fitted by double exponential (modified from [52]).

mine a functional signature of the latter. For example, coexpression of recombinant flip and flop forms of AMPAR subunits leads to formation of the ensemble channels with intermediate desensitization properties [30]. Thus one can expect that excess of mRNA expression for flop forms in a particular neuron will result in synaptic receptors with primarily flop properties. Coexpression of the edited form of the GluR-B subunit with other AMPAR subunits results in a mosaic of channels with a wide range of Ca^{2+} permeability and polyamine sensitivity [28, 35, 38]. In neurons these properties are dependent on a relative abundance of GluR-B mRNA compared with that for the other subunits [39].

Native NMDAR channels are most probably composed of NR1 and one or more NR2 subunits [1] or the recently reported NR3A subunit [9]. Studies on recombinant NMDAR channels have shown that voltage-dependent Mg^{2+} block and deactivation kinetics of ensemble channels strongly depends on subunit composition. Thus, NR1-NR2A and NR1-NR2B channels exhibit a stronger voltage dependence of Mg^{2+} block than that of NR1-NR2C and NR1-NR2D channels. In addition, deactivation kinetics of the glutamate-evoked current, which shapes the slow component of the glutamatergic synaptic responses, differs between the subunit combinations being the fastest for NR1-NR2A and the slowest for NR1-NR2D channels (fig. 2A) [40]. Kainate receptors may also form heteromeric channels with a wide range of properties. Thus, upon coexpression of Q- and R-form GluR6 subunits, the R form dominates in determining Ca^{2+} permeability and polyamine sensitivity of the ensemble channels. Some of the KAR subunits, like KA2 or KA1, do not form functional receptors by themselves, but when coexpressed with GluR5 or GluR6 subunits, they alter the properties of the latter ones [41].

Examples described above point to the importance of the next level of regulation of synaptic receptor function—formation of functional receptors in particular synapses. The appearance of functional glutamate receptors in particular synapses is also genetically regulated. It is determined by relative level of gene expression, receptor assembly and synaptic targeting mechanisms. This level of regulation of receptor function seems to be of great importance since it determines the ultimate properties of the receptor channels in a functioning synapse.

Regional- and age-specific expression of GluR subunits mRNAs in CNS cells

In situ hybridization and reverse-transcriptase polymerase chain reaction (RT-PCR) studies have shown that expression of GluR genes in brain exhibits regional and cell-specific distribution. For example, the AMPAR GluR-B subunit is preferentially expressed in principal neurons (e.g. pyramidal neurons in the CA1, CA3 regions of the hippocampus, and cerebellar Purkinje cells) and to a lesser extent in interneurons (hippocampal basket cells and interneurons in the neocortex). Importantly, the relative expression level of the GluR-B mRNA correlates with the functional identity of AMPAR channels. In most of the principal neurons high expression of GluR-B mRNA correlates with low Ca^{2+} permeability of the AMPAR channels that mediate glutamate-evoked currents. On the other hand, low expression of GluR-B mRNA in most of the interneurons correlates with high Ca^{2+} permeability of the AMPAR channels [39]. Moreover, the expression pattern of AMPAR splice variants in brain is age dependent. Thus, mRNAs for AMPAR flip forms are expressed throughout embryonic and postnatal life and remain largely invariant during postnatal development, whereas flop mRNA expression starts at a very low basal level and appears increasingly between P9 and P12 [42].

In situ hybridization at the X-ray level has revealed that NMDAR NR1 subunit mRNA is ubiquitously present at a high level in virtually all rat brain regions and both in embryonic and postnatal ages, whereas expression of NR2 subunits shows different developmental and regional distribution [40]. NR2B and NR2D subunits are expressed in embryonic CNS structures, whereas NR2A and NR2C subunits are barely detectable at this stage. They gradually come into play starting from early postnatal ages and reach the peak of expression in adulthood (NR2A in most brain regions; NR2C preferentially in cerebellum). In contrast, NR2D mRNA is practically not detectable in the brain around this time. NR2B expression peaks at P12 and decreases in adulthood in cortex, and is not detectable in cerebellum, where it is replaced by the NR2C subunit [40].

KAR subunits and their spliced and edited forms are also distributed in CNS cells in a regional- and age-dependent manner (reviewed in [29]). Thus, differential expression of mRNAs coding different GluR subunits provides a possibility to recruit a particular subunit for a particular function in synapses.

Receptor assembly

Native GluR receptors are presumably heteromeric channels composed of several subunits of the same family. The exact composition of the functional receptors is not known. It is still not unequivocally established whether GluR channels are of tetrameric or pentameric structures (discussed in [3]). It is also not known whether native receptors are homomers, doublets or triplets or composed of more subunit types. It has been shown in adult rat cerebral cortex that native NMDAR channels are mainly composed of three subunits forming heteromeric channels NR1/NR2A/NR2B with a few dimeric NR1/NR2A and NR1/NR2B channels [43]. This study hinders direct usage of the data obtained from studies of recombinant channels in which channels composed of one or two subunit types were usually investigated. The exact mechanisms by which particular subunits target a particular synapse have not yet been identified. Recent studies have shown that synaptic localization of AMPAR channels is developmentally regulated. In the CA1 region of the hippocampus at early postnatal state P2–P10 a significant fraction of excitatory synapses lack AMPAR. Later in development AMPARs are progressively added to the synapses [44]. Moreover, AMPARs localized in the dendrites may be delivered to the synaptic spines during tetanic stimulation. This activity-dependent enhancement in a number of synaptic AMPARs which requires activation of NMDAR channels may underlie long-term potentiation, at least in part [45]. Several studies (discussed in this issue) may shed light on the mechanism of GluR targeting and clustering on the surface of the postsynaptic membrane. It has been found that GluRs interact with a family of membrane associated-synaptic proteins that might be important for targeting and anchoring receptors to specific synapses.

Differential expression of GluR subunits and synaptic transmission

The functional signature of the receptors mediating synaptic transmission still remains the only true criteria for identifying specific receptors in particular neuronal circuit. However, excessive information of the expression pattern of GluR mRNAs and identified functional properties of particular subunits help to specify which function of which subunit might be important for the functioning profile of synaptic transmission in a particular neuronal circuit.

For example, in rat neocortex regular spiking pyramidal cells, 92% of GluR1–4 mRNAs are flip variants, whereas in fast-spiking (FS) nonpyramidal cells 90% of GluR1–4 mRNAs are flop variants [46]. The single-cell RT-PCR data correlate strongly with faster desensitization kinetics of the glutamate-activated currents recorded from outside-out patches pulled from the soma of the FS cells. Deactivation kinetics of the gluta-

mate-activated currents was not dramatically different between these two cell types (fig. 3A). Although somatic and synaptic AMPARs might be not the same, one can expect that in synapses that express predominantly flop variants of AMPAR subunits fast synaptic current might be terminated by desensitization of AMPAR channels. Combined with slow recovery from desensitization, it may contribute to synaptic depression during high-frequency activity of the synapses.

In most of the interneurons, for example, somatic and synaptic AMPAR channels are highly Ca^{2+}-permeable and show a sigmoidal current-voltage relation in the presence of endogenous intracellular polyamines (fig. 3B). These properties of AMPAR channels appear to be functionally very important in synaptic transmission. Activity-dependent relief from polyamine block of Ca^{2+}-permeable AMPAR channels induces facilitation of glutamate-activated currents (fig. 3B) [47]. In neocor-

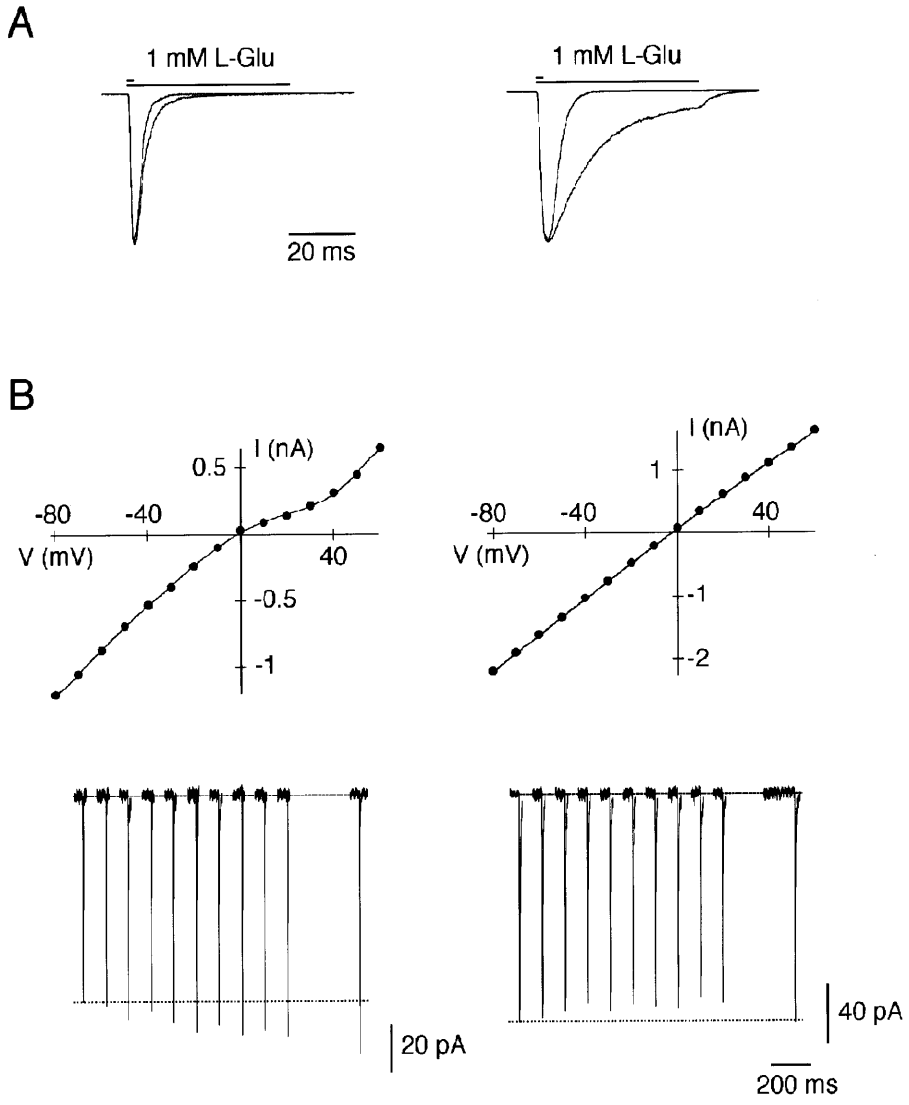

Figure 3. Diversity of AMPAR subunits expressed in different types of neurons underlies different properties of glutamate-activated currents. (*A*) Glutamate-activated currents recorded from nucleated patches pulled from parvalbumine positive fast-spiking multipolar interneurons (left) and pyramidal (right) cells of layer 2/3 rat neocortex. Duration of glutamate pulses were 1.8 or 50 ms. According to desensitization kinetics AMPAR expressed in soma of multipolar and pyramidal cells were predominantly in flop and flip forms, respectively. (*B*) AMPAR channels expressed in dentate gyrus basket cells are polyamine-sensitive (left I–V, measured from nucleated patches) and undergo polyamine-dependent facilitation in response to a train of brief glutamate applications (left trace, measured from outside-out patches). AMPAR channels in CA1 pyramidal cells due to high expression of GluR-B subunits are polyamine insensitive (right I–V) and do not undergo polyamine-dependent facilitation under the same experimental conditions (right trace).

tical interneurons this mechanism counteracts synaptic depression. The effect is more pronounced at higher stimulation frequencies at which synaptic signal filtering that occurs due to presynaptic depression or postsynaptic AMPAR desensitization is more prominent. At certain stimulation frequencies polyamine-dependent facilitation may induce short-term synaptic facilitation [48]. Thus, preferential expression of polyamine-sensitive AMPAR channels may be used by postsynaptic neurons to encode presynaptic activity. Calcium entry through synaptic Ca^{2+}-permeable AMPAR channels (although not so massive as through NMDAR channels [49]) may, in addition, induce long-lasting changes in synaptic efficacy. In the basolateral amygdala where glutamatergic synaptic transmission is mediated solely by Ca^{2+}-permeable AMPAR channels, tetanic stimulation of inputs to interneurons caused induction of long-term potentiation (LTP), which required a rise in postsynaptic Ca^{2+} concentration [50]. Hence Ca^{2+}-permeable, polyamine-sensitive type AMPAR subunits expressed in synapses in at least some interneurons may serve for activity-dependent postsynaptic control of synaptic gain in short- and long-term time scales.

Several studies reported that in central neurons the duration of NMDAR-mediated synaptic responses (which is determined mainly by NMDAR-mediated current deactivation) is shorter in older animals (23–35 days) than in younger ones (10–15 days) [51, 52]. These synaptic data correlate with a developmental switch in the cortex in expression of NR2B, NR2D subunits with a slower deactivation kinetics to the NR2A subunit, which imparts a faster deactivation kinetics (fig. 2).

Although many KAR subunits have been cloned and characterized, the functional role of KAR channels in the nervous system was not quite clear for a long time. Only recently it has been shown that this class of receptors may mediate synaptic transmission. Synaptically activated KARs have been described in mossy fiber-CA3 neuron contacts [53, 54]. Further studies revealed that in these synapses KAR channels contain at least GluR5 and GluR6 subunits [55, 56]. In the hippocampal CA1 region, activation of presynaptic kainate receptors may downregulate GABAergic inhibition of CA1 pyramidal neurons [57].

Concluding remarks

A brief overview of the levels of the genomic control of GluR function shows that ultimate properties of GluRs in certain neuronal circuits are dependent on many parameters. In situ hybridization and single-cell RT-PCR studies combined with rapid agonist application techniques using excised patches from identified neurons provided information about which subunits are expressed in specific cell types, and in some cases, gave information about the functional properties of the native somatic and dendritic GluRs. Simultaneous whole-cell recordings from pairs of synaptically connected neurons in combination with antibody or fluorescence labeling of GluRs in synaptic contacts appears to be a method of choice for functional identification of GluRs in intact synapses.

An additional promising approach has been engineering mice with genetically manipulated GluRs. Several unexpected aspects of GluR function have been found using transgenic mice with knocked-out or modified subunits. Mice deficient in NMDARs demonstrated the importance of this receptor for neuronal development and plasticity [58–61]. Gene-targeted mice lacking the GluR2 AMPAR subunit exhibit enhanced LTP in CA3–CA1 synapses in the hippocampus [62]. In mice expressing the unedited form of the GluR-B subunit, NMDAR-independent LTP might be induced in hippocampal CA3-CA1 synapses [63]. On the other hand, mice lacking the AMPAR GluR-A subunit showed no associative LTP in CA3–CA1 synapses in the hippocampus [64]. These results indicate that AMPAR channels contribute to synaptic plasticity to a higher extent than was previously anticipated. This contribution depends on the subunit composition of native receptors.

One astonishing problem with the 'transgenic' approach is that in many cases genetically manipulated mice die prenatally or early after the birth. This suggests the importance of GluRs in development but limits application of such studies for better understanding of GluR function in the mature brain. Hopefully, inducible gene targeting in a particular brain region at later developmental stages will provide a useful tool for studying peculiarities of synaptic GluRs and their role in the functioning of developed neuronal circuits.

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